

**CONTRA COSTA COUNTY BOARD OF SUPERVISORS'
TRANSPORTATION, WATER AND INFRASTRUCTURE COMMITTEE**

**Record of Meeting
1:30 PM, Thursday, May 5, 2013**

Chair Andersen and Vice-Chair Piepho were in attendance.

1. **Introductions.** (See attached signup sheet).
2. **Accept public comments on any item under the jurisdiction of the Committee and not on this agenda.** Shirley Shelangoski provided comment re: integrated pest management (See attachment).
3. **Review record of meeting for April 4, 2013.** The record was approved as submitted.
4. **Receive update on state transportation legislative activities and take action as appropriate.** (John Cunningham, Department of Conservation and Development[DCD]) Mark Watts, the County's Legislative Advocate provided an overview of items of interest to the Committee.
5. **Receive legislative report on water issues and advise as appropriate.** (Ryan Hernandez, Conservation and Development). DCD staff reviewed legislative items of interest to the Committee. With consultation of the appropriate parties, DCD staff will determine when it would be appropriate to bring the following recommendations to the Board of Supervisors: AB 378 (Hueso) SUPPORT, AB 763 (Buchanan) SUPPORT, AB 803 (Gomez) WATCH, AB 1200 (Levine) WATCH (remain neutral unless Napa and Sonoma County request us to support bill), SB 735 (Wolk) WATCH.
6. **Receive report on the Iron Horse Corridor Advisory Committee Bylaws and recommend the bylaws for adoption by the Board of Supervisors.** (Carrie Ricci, Public Works Department [PWD]) PWD staff reviewed the report and clarified the changes being recommended are: **1)** appointments are now made by District II and District IV Supervisors and staff recommends that these appointments go to the full Board of Supervisors for approval, and **2)** term of the appointment to the Advisory Committee to be changed from a two year term to a four year term. PWD staff anticipates bringing the recommendations to the Board of Supervisors in June.
7. **Receive attached report and recommend setting a public hearing before the Board of Supervisors on adoption of the proposed franchise ordinance and fee resolution.** (Carrie Ricci, Public Works Department) Tom Geiger and Eric Gelston from the County Counsel's office were present for this item. PWD staff requested, and the Committee agreed, to continue this item to the next Committee meeting in order to allow further communication and coordination with the utility companies who have additional comments.
8. **Receive Integrated Pest Management Program (IPM) Report.** (Tanya Drlik, Health Services Department) Susan JunFish provided comment and handouts (See attachment). The Committee received the report with the following direction given to HSD staff: **1)** include additional detail on pesticides (quantities, additional breakdown) in the annual report, **2)** continue outreach to other cities on IPM policies, and **3)** report on bed bug success to the Baldocchi family
9. **Receive and consider draft comment letter on the Association of Bay Area Governments (ABAG) and the Metropolitan Transportation Commission's (MTC) DRAFT - Plan Bay Area: Strategy for a Sustainable Region and take action as appropriate.:** (Patrick Roche, Conservation and Development) Per the Better Government Ordinance the Committee unanimously voted to waive the time limits under §25-2.206 in order to receive and act on material provided by staff (see attachment). DCD staff will bring recommended comments to the Board of Supervisors on May 14, 2013 on 1) The *Plan Bay Area*, and 2) the associated Environmental Impact Report. DCD staff presented the draft comment letter with the following comments from the Committee for inclusion in the letter, **1)** confirm that cities and counties retain the authority to determine housing policies, **2)** that housing does not have to a) be in a Priority

* This meeting record is provided pursuant to Better Government Ordinance 95-6, Article 25-2.205(d) of the Contra Costa County Ordinance Code.

Development Area, or b) be of a certain character, **3)** urge MTC & ABAG to ensure that transportation funding be used for transportation projects, **4)** raise the potential conflict with increasing housing densities in areas with poor air quality.

10. Adjourn to the next meeting scheduled for June 12, 2013 at 11:00 AM in Room 101.

Attachments

- A. Sign in Sheet
- B. Written Public Comment (Agenda Item #2)
- C. Written Public Comment & Handouts (Agenda Item #8)
- D. Draft Letter to ABAG/MTC Re: Plan Bay Area

Transportation, Water and Infrastructure Committee Meeting

May 2, 2013

SIGN-IN SHEET

Name	Representing	Phone
John Burgh	CCWD	688-8024
Brian Balbas	CCCPWD	313-2284
Tanya Dotlik	HSD tdotlik@hisd.cc county.us	335-3214
Carrie Ricci	PW	313-2235
Julie Bueren	PW	313-2201
Mark Seedall	CCWD	688-8119
John Cunningham	DCD TWT	674-7833
Michael Kent	CGHS	313-6587
Patrick Roche	DCD	674-7807
Ryan Hernandez	DCD	674-7879
Shirley Shelongski	PFSE	917-4855
Susan Jun Fish	PFSE	283-4609
MARK WATTS	SWM	916-446-5508
RAND REYNOLDS	CPL CHEVRON Pipeline Co.	925753-2002
John Lynn Smith	REED/Smith - KINDER MORGAN	456594863
Jill Ray	BOS DIST 2	957-8860

May 2, 2013

To: Transportation, Water and Infrastructure Committee

Public Comment from Shirley Shelangoski, Parents for a Safer Environment

Dear Honorable Chairman Candace Anderson and Honorable Mary Phiepho:

I request a clarification of the county's policy regarding the future reporting of rodenticides in our county for the Annual Report. At the Integrated Pest Management Advisory Committee meeting yesterday, I was present and heard that the staff intends to report only products listed in the PANNA database. Although all rodenticides would still be tracked by the IPM coordinator since it is required by law to provide this information to the local County Dept of Agriculture, "reporting" of rodenticides and other products not listed in the PAN database would stop.

This is not acceptable. ALL rodenticide usage must be identified, and reported in the annual report. The PAN database lists all active ingredients and their associated toxicity. The PAN database only lists federally registered products and not state registered products, because it would be require a much larger capacity than they can handle to cover each state. There are hundreds of rodenticides registered in California and they are slightly different from those registered in other states. One needs to consider the active ingredients, diphacionone and its toxicity since there are many state registered rodenticides that are not registered for sale at the federal level. Furthermore, rodenticide usage must be reported separately from herbicides because it is much more concentrated and designed to kill with miniscule exposure. Combining the application of rodenticides with the thousands of pounds of herbicides is like adding apples and chickens. They have very little in common and reporting their usage in the same mass and in the same breath provides no useful information, and actually clouds transparency.

The 2012 IPM Annual Report omits any mention of applications of rodenticides in parks by contractors of the Public Works' Special District. There are at least a dozen applications known to have been placed in public parks. A review of the Annual report would not give a hint of this. This presents a clear danger to children, pets and wildlife. A presentation was given by Nancy Wenninger, Chairman of the Conservation League at the IPM meeting yesterday citing published statistics that over 10,000 children were poisoned last year by rodenticides.

I thank you for your attention to these matters of safety for our community and ask that you have staff clarify their plans on reporting rodenticide applications. It is truly sad that instead of discussing the alternatives to rodenticides, we are discussing whether or not the staff should even report rodenticide usage in its reports.

Shirley Shelangoski


Pleasant Hill resident

sjs_159@yahoo.com

May 2, 2013

Public Comments from Susan JunFish, Director of Parents for a Safer Environment

Good Afternoon Supervisors Anderson and Piepho:

I would first like to clarify to Supervisor Piepho that neither I nor anyone else from PfSE were invited to meet with the IPM Coordinator in 2012 to get our concerns addressed, neither in the summer nor the Fall as you inferred at the December 6th TWIC meeting when 11 community members presented concerns about the county's IPM program that is not following the county's written policy. I sent several e-mails asking Ms Drlik about which meeting she was referencing but received no response to date.

We appreciate the structural pest control program that does not use rodenticide for rodents getting into buildings thanks to Pestec who we advocated to hire in place of Orkin in 2009.

But we can't ignore the glaring problems of rodenticides being used in county parks in Alamo, and throughout the county. But how can the IPM Advisory Committee, TWIC, or the community red flag this concern when over a dozen rodenticide applications in county parks were not reported in the annual report? It's bad enough that applications are rounded off to "0" on the spreadsheets used by staff, but it's another policy matter when rodenticides are suddenly being used in county parks without anyone knowing about it except staff until PfSE asked for public records to discover this.

We not only expect that all locations that are treated be correctly reported in the 2012 annual report but also that treatments are posted at the sites and also on the IPM web page. I ask you today for the record, will you do something about it?

Diphacinone, along with other county pesticides being used (including Triclopyr, Aluminum Phosphide, bromadiolone, sodium nitrate, esfenvalerate), is among those active ingredients listed on the US EPA Injunction list. This means that it is illegal to use these products in areas where there is endangered or protected species. PfSE expects the county to not leave out information in the annual report about these very risky products. Please incorporate these omissions into the 2012 annual report and in future reports.

Finally, why is it that staff get instructions from supervisors who instruct them on where and when to treat weeds with herbicides along roads and creek banks and open space but the county cannot provide the public with a list of the treated areas? It seems like a simple enough request to highlight the roads, creekbanks, and empty lot locations on a map online so the community has a choice to avoid these areas when spraying is occurring. Even the County's Mosquito and Vector Control District does this online. Why can't our county departments do this also for transparency sake?

I am also submitting for the record, 2 major studies showing that diphacinone, the active ingredient in the 32,000 lbs + of poisoned grains scattered above ground by Dept of Ag is indeed highly dangerous to birds of prey and mammals.

Thank you for your time and I would appreciate your response to my questions.

Susan JunFish, MPH
junfish@gmail.com or 925-283-4609

Susan JunFish's summary of Bad Actor presentation to IPM Advisory Committee on March 7, 2012. Submitted to Tanya Drlik on May 12, 2012 due to the information missing in the meeting minutes.

1. Bad Actor Pesticides is a term referenced by County PEHAB and Contra Costa County staff using reporting pesticide usage.
2. Bad Actor Pesticides is a term coined by Pesticide Action Network, North America, an advocacy organization based in S.F. They have been recognized and given an award for their pesticide database by the US EPA, the federal regulatory agency for pesticides.
3. There are currently 5 categories of highly toxic pesticides coined "bad actors."
 - a. known or probable human carcinogens. B. neurotoxins as acetyl choline esterase inhibitors. C. reproductive/developmental toxins. D. known groundwater contaminants. E. Acutely toxic chemicals designated w/ the "Danger" label on the product.
4. The 5 categories of highly toxic pesticides (a, b, c, d, and e) or "bad actors" have been determined by federal and state regulatory agencies. The data is streamed into PANNA's database as found in the official governmental databases.
5. CC County has missed many acute toxic pesticides that should have been included among the bad actors reported. PANNA designates these by stating that they have a "danger" label in the database, but not necessarily providing a skull bone in the bad actor column.
6. CC County has missed salts of bad actor pesticides. Most studies have been done using parent chemicals that are easier to administer quantitatively (diluted in water they become parent from salts) to animals or tissues cultures. PANNA considers salts of bad actor parent chemicals, a bad actor, but strictly relays what's in the regulatory agencies' databases.
7. Chemists Dr Susan Kegley and Dr Hal Sternberg both agree that we should consider salts of parent chemicals, the same as parent chemicals once the salt forms are diluted in water, just like the county staff dilutes pesticide products prior to application.
8. PfSE asks the county to correctly include acutely toxic pesticide products and salts of bad actor pesticide products as Bad Actor products that are being applied by county and report a complete and more accurate picture to the Board of Supervisors and to the community.
9. Currently there is a 20-35% under-reporting of Bad Actor pesticide products being used by the county,.

Anticoagulant Exposure and Notoedric Mange in Bobcats and Mountain Lions in Urban Southern California

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LYNN WHITED, *California Wildlife Center, P.O. Box 2022, Malibu, CA 90265, USA*

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ABSTRACT Humans introduce many toxicants into the environment, the long-term and indirect effects of which are generally unknown. We investigated exposure to anticoagulant rodenticides and evaluated the association between notoedric mange, an ectoparasitic disease, and anticoagulant exposure in bobcats (*Lynx rufus*) and mountain lions (*Puma concolor*) in a fragmented urban landscape in southern California, USA. Beginning in 2002, an epizootic of notoedric mange, a disease previously reported only as isolated cases in wild felids, in 2 years reduced the annual survival rate of bobcats from 0.77 (5-yr average) to 0.28. Anticoagulants were present in 35 of 39 (90%) bobcats we tested, multiple compounds were present in 27 of these 35 (77%), and total toxicant load was positively associated with the use of developed areas by radiocollared animals. Mange-associated mortality in bobcats showed a strong association with anticoagulant exposure, as 19 of 19 (100%) bobcats that died with severe mange were also exposed to the toxicants, and for bobcats with anticoagulant residues >0.05 ppm, the association with mange was highly significant ($\chi^2 = 10.36$, $P = 0.001$). We speculate that concomitant elevated levels of rodenticide exposure may have increased the susceptibility of bobcats to advanced mange disease. Bobcats were locally extirpated from some isolated habitat patches and have been slow to recover. In 2004, 2 adult mountain lions died directly from anticoagulant toxicity, and both animals also had infestations of notoedric mange, although not as advanced as in the emaciated bobcats that died with severe disease. Two other mountain lions that died in intraspecific fights also exhibited exposure to 2–4 different anticoagulants. These results show that the effects of secondary poisoning on predators can be widespread, reach even the highest-level carnivores, and have both direct and possibly indirect effects on mortality. Further research is needed to investigate the lethal and sub-lethal effects of anticoagulants and other toxicants on wildlife in terrestrial environments. (JOURNAL OF WILDLIFE MANAGEMENT 71(6):1874–1884; 2007)

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KEY WORDS anticoagulant rodenticides, bobcat, fragmentation, mountain lion, multiple stressors, notoedric mange, southern California, synergistic effects, toxicology, urbanization.

The conversion of land for urban or agricultural uses has obvious impacts on natural populations by eliminating, fragmenting, and altering habitat. However, human activity, including the introduction of toxicants into the environment, can have other unintended and more cryptic consequences for wildlife populations. Determining the nature and extent of these effects can be difficult, particularly if multiple stressors are involved. In recent years, laboratory and artificial pond experiments on aquatic amphibians have revealed that anthropogenic stressors can interact with natural ones to have a much greater influence on survival, growth, and population persistence than either factor in isolation (Kiesecker et al. 2001, Kiesecker 2002, Relyea 2003). However, there have been few similar demonstrations in terrestrial systems or in more natural field situations (e.g., Gervais and Anthony 2003).

Anticoagulant rodenticides are widely used in both urban and agricultural settings to control rodent populations.

There are 6 anticoagulant rodenticides registered by the Environmental Protection Agency for control of rats and mice in and around buildings. They are often formulated as grain-based food baits, typically pellets, although other formulations are used, and all are sold over-the-counter and are therefore available to the general public. Warfarin, chlorophacinone, and diphacinone were developed earlier and are referred to as first-generation anticoagulants. They generally require multiple feeding by the target species and are less toxic to birds. They are also more readily metabolized and are much less persistent in the body. The other 3 anticoagulants, brodifacoum, bromadiolone, and difethialone, are commonly referred to as second-generation anticoagulants. They exhibit very high toxicity to birds and mammals, can provide a lethal dose in a single feeding, and can remain in body tissues for long periods (months) because they are highly persistent and are not readily metabolized (Eason et al. 2002, Erickson and Urban 2004, Berny et al. 2006).

Secondary anticoagulant poisoning of nontarget animals has been well-documented in a wide range of birds and mammals (Eason and Spurr 1995; Stone et al. 1999, 2002;

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Hosea 2000; Eason et al. 2002) including owls (Mendenhall and Pank 1980), buzzards (Berny et al. 1997), coyotes (*Canis latrans*; Riley et al. 2003), feral cats (*Felis catus*; Alterio 1996), mountain lions (*Puma concolor*; Littrell 1988), otters (*Lutra lutra*), endangered European mink (*Mustela lutreola*), and polecats (*Mustela putorius*; Fournier-Chambrillon et al. 2004). However, the vast majority of this poisoning undoubtedly remains undetected for several reasons. Most importantly, testing for the presence of anticoagulants requires necropsy and analysis of liver tissue (using high-performance liquid chromatography [HPLC]). Unless an animal is being tracked through radiotelemetry, finding deceased animals in a nondecomposed state is rarely possible in the field (Wobeser 2006). Another problem is that many animals do not exhibit any outward signs of poisoning, so toxicants go undetected without specific testing (e.g., McDonald et al. 1998).

When testing is done, anticoagulant occurrence is often high. Shore et al. (1996) found that 31% of polecats tested in Britain had anticoagulants present, Hosea (2000) found that 70% of the mammals (including coyotes and bobcats [*Lynx rufus*]) tested in California, USA, had been exposed to anticoagulants, and anticoagulants were detected in 49% of the raptors tested in New York, USA, from 1998 to 2001, including in 81% of the great horned owls (*Bubo virginianus*; Stone et al. 2002). Although some predators die directly from anticoagulant toxicity, many others do not. For example, 86% of the raptors exposed to anticoagulants in New York did not show evidence of death from direct toxicity. However, according to Stone et al. (2002:37), "the impact of anticoagulant exposure must extend well beyond those cases in which acute lethal hemorrhage is the proximal cause of death." Little is known about what constitutes a lethal dose for wildlife species, what the sub-lethal, chronic effects may be, or what kinds of interactions may occur between anticoagulants and other factors.

We investigated the association between anticoagulants and the natural stressor of disease, specifically notoedric mange. Mange is not an uncommon disease in carnivore populations, but most reports of population-level effects are from sarcoptic mange infestation, often in canids (Pence and Ueckermann 2002). Notoedric mange has generally only been reported as isolated cases in wild felids (e.g., Pence et al. 1995, Ryser-Degiorgis et al. 2002) including both bobcats (Penner and Parke 1953, Pence et al. 1982) and captive Florida panthers (*Puma concolor coryi*; Maehr et al. 1995), although there are reports of epizootics in coatis (*Nasua narica*; Valenzuela et al. 2000) and in a feral cat population in Florida, USA (Foley 1991). The mite, *Notoedres cati*, occurs as a treatable ear parasite in domestic cats and may be more common in veterinary practices in Southern California (Brooks 2000), although it occurs in urban areas throughout the United States (K. Kwochka, DVM Pharmaceuticals, personal communication). Mange cases may become severe and result in dehydration, emaciation, and eventually death (Pence and Ueckermann 2002). However, by itself, the occurrence of mange mites,

including *Sarcoptes*, on a healthy animal would not be considered fatal but can develop into lethal disease when other factors are also present (Samuel 1981). Consequently, the prevalence of mange increases in times of increased environmental stress such as drought or winter or in animals under social or nutritional stress (Pence and Ueckermann 2002).

Following results from previous research about the occurrence of anticoagulant toxicity in coyotes (Riley et al. 2003) and more recent observations of mange-associated mortality in bobcats, we began investigating anticoagulant exposure and the potential for interactive effects of this exposure in bobcats and mountain lions. Specifically, we measured the frequency and amount of anticoagulant exposure and notoedric mange incidence in both species, and we assessed the potential association between anticoagulant exposure and susceptibility to severe mange infestation. We also evaluated the degree to which anticoagulant exposure was related to the use of development by radiocollared bobcats and the impact of the mange epizootic on the local bobcat population.

STUDY AREA

Our study was conducted in the coastal mountain ranges north and west of the city of Los Angeles in southern California, including the Santa Monica Mountains, Simi Hills, and Santa Susana Mountains (Fig. 1). The area had a Mediterranean climate with cool, wet winters and hot, dry summers. Predominant habitat types included mixed chaparral, coastal sage scrub, oak woodland and savanna, riparian areas, and introduced annual grasslands. Human land-uses included commercial development, low- to high-density residential development, golf courses, landscaped areas in parks and adjacent to office buildings, agricultural land, and a 120-ha landfill. An 8–10-lane freeway (United States Route 101), 2 4–6-lane freeways (State Routes 23 and 118), and numerous secondary roads intersected our study area (Fig. 1). For bobcats, we focused on the southern Simi Hills area, including habitat fragments, and the portions of the Santa Monica Mountains immediately across the 101 freeway (see bobcat study area, Fig. 1). For mountain lions, our study area included all of the Santa Monica Mountains, Simi Hills, and Santa Susana Mountains.

METHODS

Bobcat and Mountain Lion Capture and Radiotracking

We captured bobcats using foothold traps and snares (1996–1998) and cage traps (2000–2004) and captured mountain lions using foot-snares, cage-traps, and hound-capture. We chemically immobilized all animals with ketamine hydrochloride and xylazine hydrochloride in a 5:1 ratio, and then weighed, measured, and marked them with ear-tags. We fitted adult bobcats with very high frequency (VHF) radiotransmitters (Telonics Inc., Mesa, AZ; Telemetry Solutions, Concord, CA; Advanced Telemetry Systems, Isanti, MN). We fitted mountain lions with combination VHF and Global Positioning System (GPS) collars (Tele-

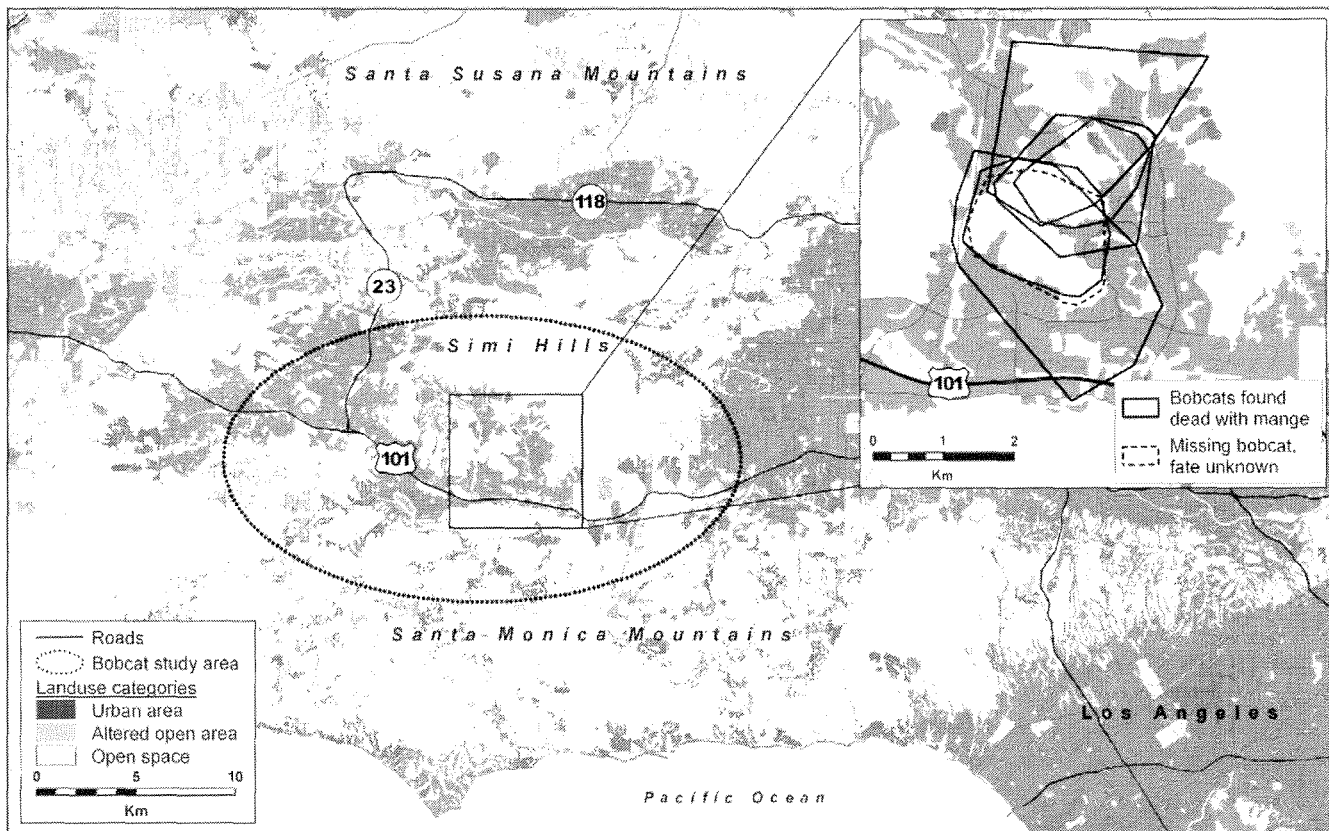


Figure 1. Study area for bobcats and mountain lions near Los Angeles, California, USA, 1996–2006. Inset: home ranges (95% min. convex polygons) of 6 bobcats in an isolated habitat patch in Westlake Village, California. Five of 6 radiocollared bobcats in this patch died of mange between March 2002 and March 2003, and contact was lost with the sixth animal. Based on trapping and scat surveys, little or no evidence of bobcat activity has been seen in this patch since.

vilt, Lindesberg, Sweden). All radiocollars were equipped with mortality sensors. We obtained the necessary permits for animal capture and handling.

We located bobcats by ground telemetry using triangulation (3 compass azimuths obtained within 15 min) 2–5 times per week throughout the 24-hour cycle. Locations used for home range analysis were separated by >12 hours. We tested radiotelemetry accuracy using test collars (see Riley et al. 2003) and it averaged 42.4 m (SD = 50.2). We calculated 95% minimum convex polygon home ranges using the home range extension in the program ArcView. We calculated bobcat survival rates with the techniques of Heisey and Fuller (1985) using the program MICRO-MORT.

We calculated the urban association (the relative amt of potential interaction with human development) of bobcats by determining the percentage of developed land and the percentage of altered land (areas that are not natural habitat, but are potentially more conducive to wildlife use than developed areas) present in the home range (see also Riley et al. 2003). Developed land included commercial areas and residential areas with ≥ 1 house per 0.4 ha. Altered land included golf courses, landscaped areas such as office or city parks, a landfill, small strips or patches of habitat, and low-density (≤ 1 house/2 ha) residential areas. We define unnatural area as developed areas plus altered areas.

We radiotracked mountain lions 2–3 times per week by VHF ground telemetry, and we obtained 100–150 GPS collar locations each month by remote download. Potential mountain lion prey kill-sites were identified by examining each month's GPS locations for clusters of locations (Anderson and Lindzey 2003) encompassing a period of 24 hours or more. Potential kill sites were investigated and prey remains were identified to species, age, and sex, when possible.

Bobcat Distribution and Relative Abundance

We collected scat on established transects each month. Over multiple years, changes in the numbers of scat collected over time allowed us to monitor changes in bobcat distribution and relative abundance. Transects were cleared in March 2001, and then all bobcat scat was collected each month through December 2004, except for 3 months in 2002. We identified bobcat scat by size and shape (Murie 1954). Verification of species identification of bobcat scat in the same region using faecal genotyping revealed approximately 90% accuracy using field characteristics (Kohn et al. 1999, Fedriani et al. 2000), although for examining trends over time, consistency of technique is most important.

Necropsy and Diagnostic Analysis

When we detected mortality signals, we immediately located the carcasses of radiocollared animals. We determined cause

of death by necropsy and, when possible, by using associated ancillary tests including routine histology, bacteriology, virus isolation, and toxicology. We transported the first 4 bobcats that showed indications of mange to the California Animal Health and Food Safety Laboratory (CAHFS) San Bernardino Branch, where full necropsies were performed. We treated skin scrapings from these animals with 10% potassium hydroxide and examined them microscopically for mites, and we examined mites as whole mounts in glycerin. We identified future cases of mange in bobcats by visual examination and by the distinctive pattern of disease progress for notoedric mange. We performed a field necropsy on the first mountain lion that died, an adult female. We transferred her liver, head, and pelt and the complete carcasses of the other 3 mountain lions, an adult male, an adult female, and a yearling female, to CAHFS for examination. We also obtained skin scrapings and mites from the 2 mange-infested mountain lions, which we processed as described for the bobcats.

Anticoagulant Testing

We collected liver samples from radiocollared bobcats that died and were recovered intact. We also collected livers from unmarked bobcat carcasses encountered in the field and from 2 bobcats collected by the City of Los Angeles. We froze all liver samples at -20°C and shipped them to the CAHFS laboratory (Davis Branch), where they were tested for the presence of 7 anticoagulants: warfarin, bromadiolone, coumatol, brodifacoum, diphacinone, chlorphacinone, and difethialone. We also collected blood samples from 3 bobcats that were afflicted with mange when live-captured. For 2 of these animals, we measured prothrombin time of blood samples to determine anticoagulant presence. These samples were analyzed by Idexx Veterinary Services (San Bernardino, CA). Normal prothrombin time was considered to be 7.0–12.7 seconds, based on values for domestic cats. The third animal was B108, a female bobcat that also had an early stage case of mange and was kept at the California Wildlife Center for rehabilitation (see Results). Partial thromboplastin time was determined for this bobcat (Idexx Veterinary Services). Between 10 seconds and 28 seconds was considered normal partial thromboplastin time, again based on values for domestic cats.

We analyzed liver samples using a previously published method for the analysis of anticoagulant rodenticides in serum (Palazoglu et al. 1998) modified for tissue analysis. We acidified 5-g liver samples with acetic acid and homogenized them with 5% ethanol in ethyl acetate. We cleaned up the samples using gel-permeation chromatography (GPC). We then exchanged the GPC eluent to methanol and reduced the volume of methanol to 0.5 mL. We analyzed the methanol extracts by HPLC using diode-array and fluorescence detectors in series. Identification was by comparison with diode-array and fluorescence spectra from known standards. In cases in which diode-array and fluorescence spectra were ambiguous, we performed qualitative confirmation analysis using liquid chromatography-mass spectrometry. We performed quantification by com-

parison of analyte response in samples with that of known standards. Minimum detectable levels were 0.05 ppm for warfarin, bromadiolone, and coumatol, 0.01 ppm for brodifacoum, and 0.25 ppm for diphacinone, chlorphacinone, and difethialone.

Statistical Analyses

We measured the association between severe notoedric mange and anticoagulant exposure using a chi-square test. We assessed the relationship between mange and urban association of radiocollared bobcats with nonparametric Mann-Whitney *U* tests, measured the relationship between the total concentration of anticoagulants (ppm) and urban association with simple linear regression, and compared the total concentration of anticoagulants between mange-infected and uninfected bobcats with a 2-sample *t*-test. We report statistical results with a *P*-value of ≤ 0.10 . We performed statistical tests with the program SYSTAT (SPSS Inc., Chicago, IL).

RESULTS

We tested the livers from 39 bobcats, including animals with and without mange and that died both before and after the mange epizootic. Anticoagulant toxicants were present in 35 of 39 (90%) bobcat livers, and 27 of these 35 (77%) revealed exposure to ≥ 2 compounds. We detected brodifacoum in 31 of the liver samples at levels ranging up to 0.56 ppm, bromadiolone in 25 livers at levels up to 0.82 ppm, diphacinone in 12 livers up to 0.58 ppm, and difethialone in 10 livers at trace levels (<0.25 ppm; Table 1). Prothrombin times for the 2 bobcats whose blood we tested were 17.3 seconds and >100 seconds (normal time: 7.0–12.7 sec). The livers of all 4 mountain lions we tested indicated high levels of the 2 compounds most common in bobcats, and one mountain lion also had significant levels of difethialone and trace levels of diphacinone. In the 2 mountain lions that died of anticoagulant toxicity (see below), we detected bromadiolone at 1.27 ppm and brodifacoum at 0.57 ppm in the liver of the male (P3) and bromadiolone at 0.51 ppm and brodifacoum at 0.31 ppm in the female (P4). Two other mountain lions were killed in August 2005 (ad F P2) and June 2006 (yearling F P7) by an adult male lion. We detected bromadiolone at 0.37 ppm and brodifacoum at 0.32 ppm in the liver of P2 and bromadiolone at 0.66 ppm, brodifacoum at 0.32 ppm, difethialone at 0.66 ppm, and diphacinone at trace levels in P7.

Mange-afflicted bobcats and mountain lions exhibited severe mite encrustation on the head and shoulders (Fig. 2) in the form of proliferative dermatitis with hyperkeratosis, epidermal scaling and multiple mites, identified as *Notoedres cati*, on the surface in keratin tunnels (see also Uzal et al. 2007). In the bobcats the mange often extended over much of the body, including the hind legs. The bobcats became emaciated and increasingly diurnal in many cases, and eventually succumbed to the disease. With one exception, bobcats that died of mange did not show evidence upon necropsy of direct anticoagulant toxicity as a cause of mortality. Specifically, we did not find evidence of a

Table 1. Anticoagulant exposure of bobcats in the Santa Monica Mountains and Simi Hills, Ventura and Los Angeles Counties, California, USA, 1997–2003. Values indicate residues from liver samples (ppm). We captured and radiocollared all bobcats with identification (ID) numbers except for B050, which had an identification collar without a radiotransmitter. All bobcats with ID letters were recovered by other means, such as being recovered on a road, submitted to an animal control agency, or seen and reported by a homeowner. We also conducted tests for warfarin, coumatichlor, and chlorophacinone, but we detected no residues except for a trace of warfarin (mdl = 0.05) in Bobcat 114.

Bobcat ID	Sex	Age	Bromadiolone mdl = 0.05 ^a	Brodifacoum mdl = 0.01 ^a	Diphacinone mdl = 0.25 ^a	Difethialone mdl = 0.25 ^a	Total ^a	Mange?	Yr	Cause
30	F	ad	nd	0.05	nd	nd	0.05	n	1997	anticoagulant toxicity
H	F	ad	nd	0.03	nd	nd	0.03	n	1988	roadkill
E	M	ad	nd	nd	nd	nd	nd	n	1988	unknown
25	F	ad	nd	nd	nd	nd	nd	n	1988	unknown
I	F	ad	nd	0.02	nd	nd	0.02	n	1988	roadkill
50	F	ad	tr	0.02	nd	nd	0.02	n	1999	unknown
G	M	ad	nd	0.01	nd	tr	0.01	n	1999	unknown
52	M	ad	0.11	0.07	nd	nd	0.18	n	1999	unknown
11	F	ad	nd	nd	nd	tr	tr	n	1999	predation
79	F	ad	0.48	0.22	nd	nd	0.70	y	2002	mange
54	F	ad	nd	nd	nd	nd	nd	n	2002	roadkill
98	F	juv	tr	0.02	0.58	nd	0.60	n	2002	roadkill
F	F	ad	nd	nd	nd	nd	nd	n	2002	roadkill
65	M	ad	0.36	0.37	nd	nd	0.73	y	2002	mange
66	M	ad	nd	0.09	0.30	nd	0.39	y	2002	mange
67	F	ad	tr	0.07	nd	nd	0.07	y	2002	mange
72	F	juv	nd	tr	nd	nd	tr	y	2002	mange
91	M	ad	0.50	0.14	tr	tr	0.89	y	2002	mange
92	F	ad	0.13	0.11	tr	tr	0.64	y	2002	mange
114	M	ad	0.08	0.44	nd	tr	0.52	n	2003	unknown
115	M	ad	0.09	0.31	tr	nd	0.40	n	2003	fence
A	M	ad	nd	0.03	nd	nd	0.03	y	2003	mange
B	M	ad	0.82	nd	nd	nd	0.82	n	2003	roadkill
C	F	ad	tr	nd	nd	nd	tr	n	2003	roadkill
95	M	juv	tr	nd	tr	nd	tr	n	2003	roadkill
116	M	juv	0.05	0.24	nd	nd	0.29	n	2003	roadkill
118	M	ad	0.10	0.02	nd	nd	0.12	n	2003	roadkill
D	M	ad	nd	0.11	0.29	tr	0.40	n	2003	unknown
7	M	ad	0.18	0.34	tr	tr	0.52	y	2003	mange
22	M	ad	0.10	0.17	nd	nd	0.27	y	2003	mange
70	M	juv	0.09	0.25	tr	nd	0.34	y	2003	mange
71	F	ad	0.14	0.36	tr	tr	0.50	y	2003	mange
76	M	ad	0.21	0.29	tr	tr	0.50	y	2003	mange
84	M	ad	0.11	0.05	nd	nd	0.16	y	2003	mange
88	F	ad	0.05	0.03	nd	nd	0.08	y	2003	mange
106	F	juv	0.16	0.24	nd	nd	0.40	y	2003	mange
112	F	ad	0.07	0.11	nd	nd	0.18	y	2003	mange
113	F	ad	tr	0.07	0.27	nd	0.34	y	2003	mange
121	M	juv	0.15	0.56	nd	tr	0.71	y	2003	mange

^a tr = positive, but amt < min. detectable limit (mdl) for that compound; nd = not detected.

coagulopathy (manifested by extensive internal hemorrhaging), indications that were seen previously in >8 coyotes and 1 bobcat that were determined to have died directly from anticoagulant toxicity (Hosea 2000, Riley et al. 2003). On the other hand, both mountain lions died directly from anticoagulant toxicity as demonstrated by the high levels of anticoagulants detected in the liver, multiple extensive hemorrhages on serosal surfaces and within body cavities, and the lack of evidence of trauma or other lesions to justify the bleeding. In addition, we obtained negative results from all the other ancillary tests performed.

Severe mange and anticoagulant exposure were highly associated as 19 of 19 bobcats with advanced mange had anticoagulant compounds in their liver, 18 at more than trace levels (Table 1). Including the 2 mountain lions, 21 of 21 wild felids (100%) with mange also had anticoagulant

toxicants present. The anticoagulant levels were also generally high in bobcats with mange, and for bobcats with anticoagulant residues >0.05 ppm, the association with mange was very high (Table 2; $\chi^2 = 10.36$, $P = 0.001$). By contrast, just 8 of 20 bobcats (40%) that died of other causes exhibited a similar level of anticoagulant exposure. Overall, the total anticoagulant level in bobcats that died with severe mange ($\bar{x} = 0.39$) was higher than in bobcats that died of other causes ($\bar{x} = 0.17$; $t = 2.67$, $P = 0.011$). There did not appear to be a specific, or threshold, level of anticoagulants in bobcats with mange because 3 of the bobcats that died with mange had 0.03 ppm, 0.07 ppm, and trace levels of anticoagulants, although it is impossible to know the level of toxicant present when the mange infestation began.

In November of 2003, we captured an adult female bobcat (B108) in the early stages of mange development that also



Figure 2. Bobcat with severe mange, Simi Hills, Ventura County, California, USA, 2002.

had a prolonged partial thromboplastin time (PTT) of 50.6 seconds indicative of anticoagulant exposure (normal time: 10–28 sec). She was held captive at a wildlife rehabilitation center (California Wildlife Center, Calabasas, CA) and treated with vitamin K₁ for anticoagulant intoxication and 2 doses of ivermectin for mange. After 2 weeks, her PTT was still elevated (>100 sec), but after 4 weeks it was normal (21.0 sec) and she had no signs of mange. We released her at the capture site in December 2003, and she subsequently produced a large litter of 4 healthy kittens in the spring of 2004. However, she was apparently exposed again to mange, and she and her surviving kitten both died with severe mange infestations in the autumn of 2004.

Beginning in 2002, when the mange epizootic began, the bobcat survival rate fell from a high of 0.847 in 1999 and a 5-year average of 0.770 to 0.280 in 2003 (Table 3). Simultaneous scat surveys also showed a significant decrease in bobcat activity starting in the autumn of 2002 (Fig. 3). Finally, local extirpations of bobcats have occurred in some of the habitat fragments in the area, both large and small. In one larger patch that is surrounded by roads and development, we were radiotracking 6 bobcats (3 F, 3 M) at the beginning of 2002 (Fig. 1, inset). All 3 males and 2 of the females died with severe mange, and we lost contact with the third female's radiocollar. Scat surveys and intensive trapping for bobcats in 2004–2006 found little evidence of bobcat activity. In 2005, 2 radiocollared male bobcats occasionally visited this patch.

For bobcats in this fragmented landscape, use of developed areas was not significantly greater for bobcats with mange (% of the home range consisting of unnatural area: Mann–Whitney $U = 234$, $P = 0.351$). Although neither mange nor the presence of anticoagulants were associated with human development, the total concentration (ppm) of all anti-

Table 2. Relationship between notoedric mange and anticoagulant exposure of >0.05 ppm in bobcats in the Santa Monica Mountains and Simi Hills, Ventura and Los Angeles Counties, California, USA, 1997–2003. Two mountain lions also had both notoedric mange and high levels of anticoagulant exposure.

Mange	Anticoagulants >0.05 ppm	
	Yes	No
Yes	17	2
No	8	12

coagulants in the livers of radiocollared bobcats was related to their use of developed areas. Total concentration was positively related to the percentage of the home range made up of developed area ($r^2 = 0.255$, $F_{1,20} = 6.85$, $P = 0.017$) or unnatural area ($r^2 = 0.163$, $F_{1,20} = 3.91$, $P = 0.062$) and to the percentage of radiolocations in developed areas ($r^2 = 0.310$, $F_{1,20} = 8.97$, $P = 0.007$) or unnatural areas (Fig. 4; $r^2 = 0.249$, $F_{1,20} = 6.64$, $P = 0.018$).

The radiocollared mountain lions were generally less urban-associated than the bobcats, but both mountain lions (P3 and P4) diagnosed with anticoagulant intoxication died after spending the bulk of their last month in the most developed parts of their home ranges. For example, just prior to his death, there were multiple locations for the male mountain lion (P3) in the same habitat fragments used by many of the bobcats, only the third month in 16 that he utilized these patches (southern part of Simi Hills, see Fig. 1). The female mountain lion (P4) lived almost exclusively in the Santa Susana Mountains (Fig. 1), a large contiguous block of open space, and only used the more developed Simi Hills area during the month before she died. The other 2 lions (P2 and P7) that were killed in intraspecific fights lived exclusively south of the 101 Freeway in the less-developed Santa Monica Mountains (Fig. 1).

DISCUSSION

We frequently detected anticoagulant rodenticides in the wild felids in this landscape; including the 4 mountain lions, 91% of the cats we tested were positive for ≥ 1 compound. Bobcats are strict carnivores and the cases were widespread geographically and temporally, so we expect that most if not all exposure is secondary, from bobcats consuming poisoned prey. The presence of multiple compounds in 27 of 35

Table 3. Survival rate of radiocollared bobcats in the Santa Monica Mountains and Simi Hills, Ventura and Los Angeles Counties, California, USA, from 1997 to 2003, showing decrease in survival rate caused by mange.

Yr	No. of bobcats	No. of deaths	No. of mange deaths	Survival rate
1997	22	3	0	0.836
1998	33	7	0	0.707
1999	19	3	0	0.847
2000	20	3	0	0.796
2001	34	8	1	0.685
2002	31	15	8	0.516
2003	25	15	10	0.280

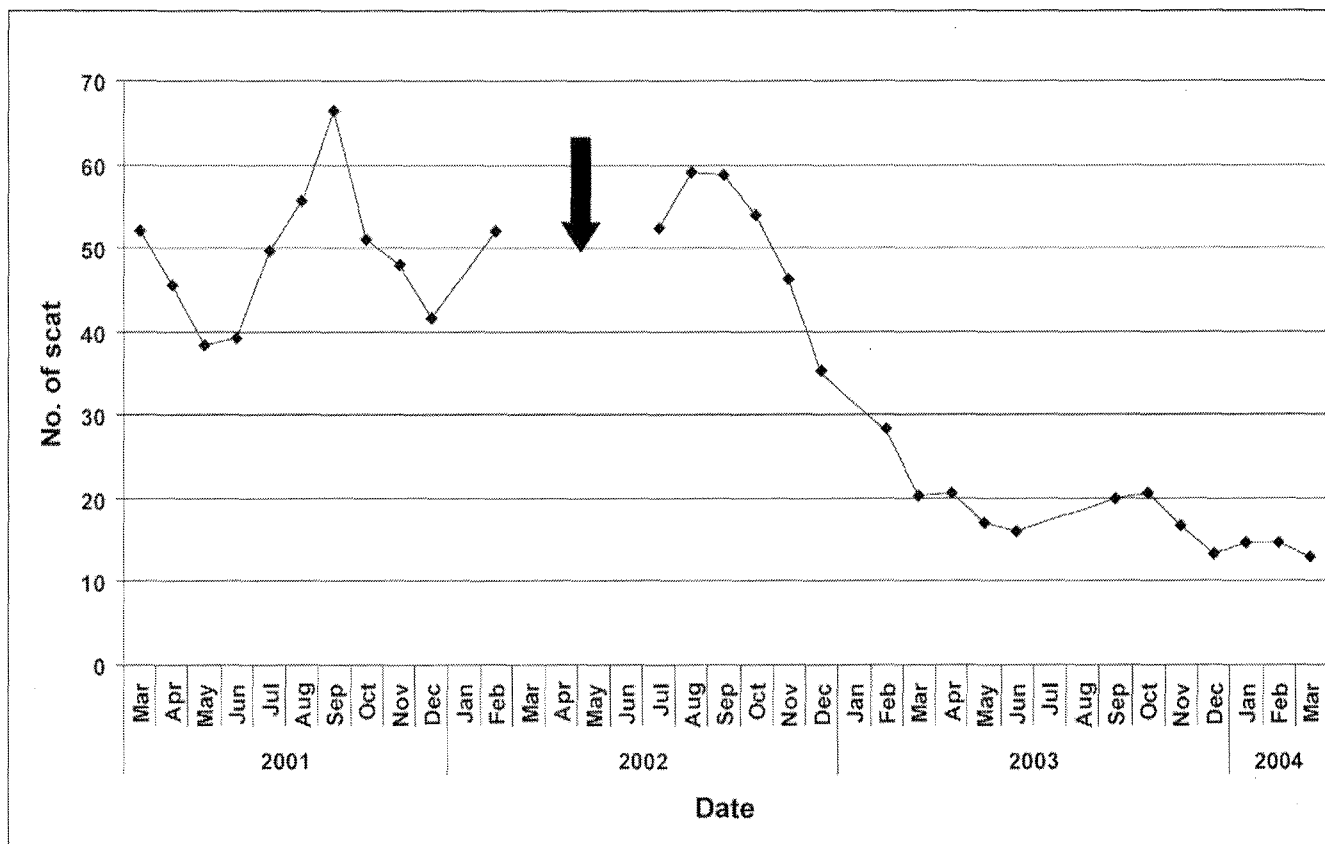


Figure 3. Three-month running average of the number of bobcat scat collected on standardized scat transects from 2001 to 2004 in the Santa Monica Mountains and Simi Hills, Ventura and Los Angeles Counties, California, USA. The arrow indicates the beginning of the mange epizootic.

exposed bobcats and all 4 mountain lions tested also points to secondary exposure and to the potential for accumulation of toxicity as a result of multiple exposure events. Anticoagulants are highly persistent in tissue (Eason et al. 2002, Erickson and Urban 2004, Wobeser 2006), with liver retention times of up to 256 days for bromadiolone, and >250 days for brodifacoum (see Eason et al. 2002, table 6 for review, references). Target rodents may also ingest a much larger than lethal dose in the days between initial bait ingestion and eventual death (Erickson and Urban 2004), in part because of the delayed onset of toxic effects (often 3–5 d) following ingestion (Murphy and Talcott 2006), thereby increasing the amount of toxicant available to a carnivore. The lack of a significant relationship between either mange or anticoagulant exposure and urban association suggests that even bobcats with low levels of development within their home ranges were close enough to developed or altered areas to be exposed to anticoagulants. Of particular concern is the finding that toxicant concentrations increased in bobcats that more frequently utilized developed areas. This is consistent with repeated exposures, and it also suggests that as development continues to encroach upon remaining habitat, anticoagulant exposure among carnivores may increase.

The compounds that we detected, brodifacoum, bromadiolone, diphacinone, and difethialone, are widely available as household and landscape rodenticides. In our study area, those known to have used anticoagulants to target rodents

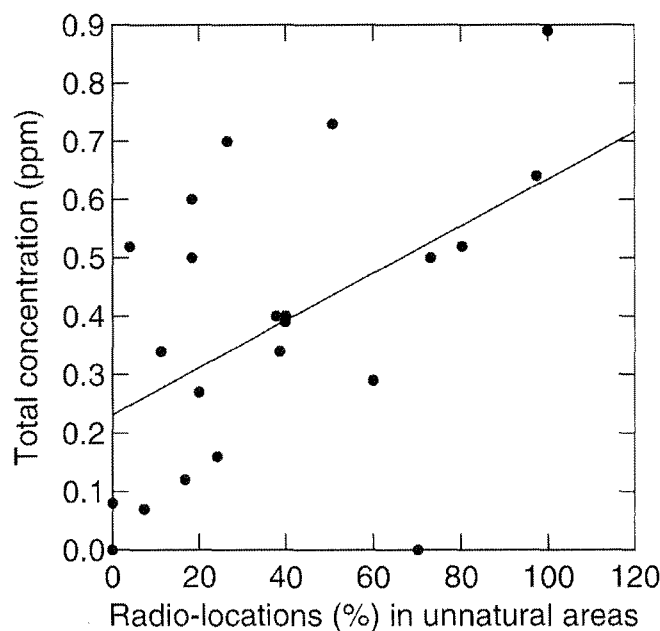


Figure 4. Linear regression of total concentration of all anticoagulants (ppm) found in the livers of radiocollared bobcats and the percentage of radiolocations for those bobcats in unnatural areas (developed + altered areas), in the Santa Monica Mountains and Simi Hills, Ventura and Los Angeles Counties, California, USA, 1997–2003.

include private homeowners, golf courses, office parks, schools, water utilities, apartment complexes, and park agencies. Brodifacoum, bromadiolone, and difethialone, which represent 67 of the 79 anticoagulant detections, are registered specifically for use in or adjacent to buildings to control commensal rodent pests, specifically Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus*). They are not designated for the control of species such as rabbits (*Sylvilagus audubonii*), woodrats (*Neotoma* spp.), pocket gophers (*Thomomys bottae*), and ground squirrels (*Spermophilus beecheyi*) that make up the dominant prey of bobcats in this area (Fedriani et al. 2000; C. S. Schoonmaker, National Park Service [NPS], unpublished data). The high prevalence of these compounds in wild carnivores suggests widespread exposure of native, free-ranging, nontarget (at least according to the regulations) species.

The deaths of 2 mountain lions as a direct result of anticoagulant toxicity (see also Uzal et al. 2007) and the exposure of all 4 lions tested indicate the pervasiveness of these toxicants across the landscape and that they are reaching the highest levels of the food chain (see also Littrell 1988). Rodents commonly constitute a significant component of bobcat diets (Anderson and Lovallo 2003), but mountain lions specialize on ungulate prey, and 157 (95.2%) of 165 known lion kills in our study (through 2004) were mule deer (*Odocoileus hemionus*). Anticoagulant residues have not been widely reported in ungulates (but see Stone et al. 1999, Eason et al. 2002), but we only recovered kills requiring ≥ 1 day to eat, so we would generally not detect smaller prey items. We did find coyotes killed by radiocollared lions, and coyotes made up 15% and 7% of the kills for the 2 lions that died of anticoagulant intoxication but only 4% of kills overall. Anticoagulant toxicity was previously documented as a leading cause of death for coyotes in this area (Riley et al. 2003), and if coyotes are retaining anticoagulants, a lion eating a coyote could ingest a large quantity of toxicant at once. Both mountain lions consumed coyotes during the last month before they died.

The association between anticoagulants and mange suggests that synergistic interactions between natural and anthropogenic stressors can occur in terrestrial ecosystems. In particular, bobcats that have been exposed to anticoagulant rodenticides appear highly susceptible to succumbing to severe mange infestations, although not all anticoagulant-exposed bobcats necessarily contract mange, presumably because not all bobcats encounter *Notoedres* mites. There has been much recent discussion in the ecological literature about the enhanced effects of multiple stressors and, in particular, of natural and human-caused stressors (e.g., Sih et al. 2004). However, the empirical work has generally been in aquatic systems and in laboratory or artificial pond settings (e.g., Mills and Semlitsch 2004, Rohr et al. 2004). Here, we have documented what appear to be significant population effects in the field.

The interaction between mange and anticoagulant rodenticides also appears to be critical because each stressor by

itself would likely not have the same impact based on available evidence and previous studies. Notoedric mange has been rarely reported in wild felids, never at epizootic levels, and never in free-ranging adult mountain lions (Uzal et al. 2007). In domestic cats, young animals and those debilitated by retroviral disease are more susceptible to mange (Sparger 1990, Guaguère 1999), suggesting that complicating factors are important in disease development. Pence et al. (1982) report cases in an adult male bobcat and a litter of kittens. Wassmer et al. (1988) found that 4 of 17 captured bobcats (24%) showed evidence of mange, one of which died of the disease. However, the other 3 bobcats had only bare patches “suggesting a current or former mild mange infestation” (Wassmer et al. 1988:176). This population was also experiencing a concurrent epizootic of feline panleukopenia virus, so this disease may have contributed to the mange incidence. Despite 5 deaths from mange (notoedric and sarcoptic) in an endangered lynx (*Lynx lynx*) population in Switzerland, Ryser-Degiorgis et al. (2002) conclude that an epidemic is unlikely because of the less social nature of felids. In our study, the appearance of severe, widespread mange in adult animals prompted us to look at other factors, including anticoagulants.

Anticoagulant poisoning alone also appears to be less of a direct threat to bobcats. Although it was the most common source of mortality in coyotes, we documented one case of potential anticoagulant poisoning in bobcats in the first 5 years of our project (Riley et al. 2003). Laboratory research on anticoagulants has determined that felids have up to 100 times greater resistance than canids to certain compounds, including brodifacoum (Roder 2001, Morgan et al. 2003, Erickson and Urban 2004). There is also previous evidence of the interactive effects of anticoagulants and other stressors in mammals. In laboratory experiments with rabbits, anticoagulant levels that produced zero mortality alone resulted in 40%–70% mortality when combined with other stressors (e.g., frostbite), and similar results were obtained with rats (Jacques 1959). In a more recent study of free-ranging merino sheep (*Ovis aries*; Robinson et al. 2005), anticoagulants had a greater effect than in a similar study of sedentary sheep, and stress (specifically shearing) in combination with anticoagulant exposure caused more mortality than anticoagulant exposure alone. Bobcats and mountain lions are certainly susceptible to direct mortality from anticoagulant toxicity as demonstrated by the deaths of 1 bobcat and 2 lions.

Although we have demonstrated a strong association between anticoagulant exposure and notoedric mange, this is not the same as establishing cause and effect. Experimental evidence would be ideal, but this kind of manipulative experiment would be logistically and ethically very difficult, if not impossible, to pursue with wild felids. Wobeser (1994), reviewing Susser (1973), discusses 5 criteria or guidelines for inferring causal relationships related to disease in wild animals: strength of association, specificity of association (one cause produces one effect), time sequence, consistency (similar results in other populations), and

coherence with current knowledge about the disease. In medicine and epidemiology, these five are sometimes broadened to nine, including biological gradient (a dose response), biological plausibility (similar to coherence), experimental evidence, and analogy (Hill 1965).

Many of these criteria will not be met even in cases where a cause does produce an effect (e.g., specificity: 1 cause can produce >1 effect, and vice versa), and they are often difficult to evaluate in wildlife populations. However, the criteria can be useful in evaluating the potential for a causal relationship, in this case whether exposure to anticoagulants causes increased susceptibility to advanced mange disease (not necessarily increased exposure to *Notoedres cati*). We believe that the strength and the specificity of the association are clear in this case, as every felid with mange was also exposed to anticoagulants, and there is a highly significant association between the two. There is also evidence of a biological gradient, as bobcats with mange had significantly higher levels of exposure to anticoagulants. We have less information for the other criteria, and there are potential alternative explanations for our results. We address both of these issues below. Further research is certainly needed to more fully resolve this question. However, we believe that the available evidence points to anticoagulant exposure contributing to advanced and fatal mange disease.

In the case of each bobcat, we do not know that the anticoagulant exposure preceded the infestation, or specifically the manifestation, of notoedric mange. It is possible that the bobcats contracted mange, the mange infestation became advanced, and the bobcats were then more likely to prey on animals contaminated with anticoagulants. Several factors argue against this alternative. If anticoagulant exposure was the result of animals with mange eating prey exposed to anticoagulants, we would not expect to find anticoagulant exposure in bobcats prior to the beginning of the mange epizootic. However, on the contrary, 7 of 9 tested bobcats that died before 2002 were positive for anticoagulant exposure (Table 1). Under this alternative, we would also not expect to find bobcats with significant anticoagulant levels that did not have mange. In fact 6 animals that died after the beginning of the mange epizootic, but had no evidence of mange, had high levels of anticoagulants. Finally, this alternative presumes that bobcats weakened by mange would eat prey weakened by anticoagulants but that healthy bobcats would not. There is no reason to believe that healthy bobcats would pass up potential prey animals, including ones that were less able to escape.

It is also possible that the mange and anticoagulant exposure were simply coincident. This alternative is also related to the consistency criterion: why has this interaction not been documented in the past? Given the paucity of studies of carnivores in urban areas, and specifically of bobcats, this is not surprising. Although other studies are underway, to date we know of 2 published studies of radiocollared bobcats in urban areas, one in the San Francisco Bay area (Riley et al. 2004, Riley 2006) and this

project. The northern California study also documented a case of mortality caused by anticoagulant poisoning (Riley 1999, Hosea 2000), although not any cases of mange. However, our study is the only long-term study of bobcats in urban areas and is, to our knowledge, the longest continuous radiotracking study of bobcats ever undertaken (E. M. Anderson, University of Wisconsin—Steven's Point, personal communication). Without intensive and long-term study, periodic changes in disease prevalence or interactions between a disease and toxicants would be very difficult to document. Moreover, although anticoagulants have been in use for a long time, the second-generation anticoagulants implicated here came into widespread use more recently (e.g., brodifacoum in the 1990s; Eason et al. 2002).

Another potential alternative is that the severe mange is related to exposure to other diseases. Advanced mange, both notoedric and sarcoptic, is generally associated with debilitation by some other factor (Samuel 1981, Pence and Ueckermann 2002), including disease. There was no evidence of other disease in the 4 bobcats upon which full necropsies (with ancillary tests, see Methods) were performed, although the general poor condition as a result of the advanced mange may have masked signs of other conditions. We have tested bobcats serologically for a number of viral diseases including feline panleukopenia virus, feline infectious peritonitis, feline herpes virus, and feline calicivirus (J. E. Foley, NPS, unpublished data), and there is no indication that animals that died of mange had greater evidence of exposure to these diseases.

Although we have not been able to investigate specific mechanisms that could be responsible for the potential interaction between mange and anticoagulants, it is certainly conceivable that anticoagulant exposure could increase the likelihood of severe mange infestation (the coherence and biological plausibility criteria). Anticoagulant poisoning causes a broad spectrum of clinical signs resulting from hypovolemia from hemorrhage, organ dysfunction, or bleeding into cavities (Searcy 2001). A sub-lethal, chronic anemia, in turn, may lead to increased susceptibility to disease and leave the animals more vulnerable to mange or other stressors. Other studies have also found sub-lethal effects of anticoagulants. For example, female sheep (*Ovis aries*) exposed to anticoagulants had more aborted or stillborn lambs (up to 50%), male sheep had lower sperm motility (Robinson et al. 2005), and barn owls (*Tyto alba*) fed difenacoum-killed rats exhibited sub-lethal haemorrhaging (Mendenhall and Pank 1980). Other chronic, sub-lethal effects have included decreased food intake in sheep (Oliver and Wheeler 1978), liver damage in brushtail possums (*Trichosurus vulpecula*; Jolly et al. 1994, Littin et al. 2002), and a decrease in body weight in brushtail possums (Littin et al. 2002). Decreases in feeding and weight in particular could affect overall condition and thereby disease resistance. Sub-lethal effects may also be more likely in a species such as bobcats that is less susceptible to direct, lethal coagulopathy than, for example, coyotes.

MANAGEMENT IMPLICATIONS

At present, anticoagulant rodenticides are seen as an effective and inexpensive method of killing rodents. However, anticoagulant applicators, including homeowners, landscape professionals, pest control operators, and land and resource managers, should consider that these chemicals can have significant impacts on nontarget wildlife. Especially in areas of high anticoagulant use such as urban areas, exposure of nontarget carnivores to anticoagulant rodenticides may be extensive and can result in direct mortality and possibly sublethal effects, potentially including complex interactions with other factors such as our data suggest. Increased awareness and the use of alternative pest control methods should reduce risks to nontarget wildlife, including carnivores. Where species of conservation concern may be exposed, further regulation of the use of anticoagulant rodenticides may be warranted. Managers should also be aware that the effects of anticoagulant rodenticides may be difficult to document without intensive study, but that a range of species may be affected. Finally, our results indicate that severe notoedric mange in bobcats, previously undocumented as an epizootic, can have population-level effects, and that those effects may be particularly significant in fragmented landscapes where local extirpations can occur.

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Comparative Toxicity of Diphacinone to Northern Bobwhite (*Colinus virginianus*) and American Kestrels (*Falco sparverius*)

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ABSTRACT: The acute oral toxicity of the anticoagulant rodenticide diphacinone was found to be about 20 times greater to American kestrels ($LD_{50}=97$ mg/kg) than to northern bobwhite ($LD_{50}=2,014$ mg/kg). Several precise and sensitive clotting assays (prothrombin time, Russell's Viper venom time, thrombin clotting time) were adapted for use in these species, and this combination of assays is recommended to detect effects of diphacinone and other rodenticides on coagulation. Oral administration of diphacinone over a range of doses (sublethal to the extrapolated LD_{15}) prolonged prothrombin time and Russell's Viper venom time within 24 to 48 hrs post-exposure. Prolongation of *in vitro* clotting time reflects impaired coagulation complex activity and was detected before or at the onset of overt signs of toxicity and lethality. These data will assist in the development of a pharmacodynamic model to assess and predict rodenticide toxicity to non-target avian species.

KEY WORDS: anticoagulant, birds, clotting time, diphacinone, fibrinogen, non-target effects, prothrombin time, Russell's Viper venom time, secondary poisoning, thrombin clotting time

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INTRODUCTION

In the past 15 years, several current use anticoagulant rodenticides have been identified as potential hazards to predatory and scavenging birds, and adverse effects have been reported in many countries (e.g., Newton et al. 1990, Eason and Spurr 1995, Howald et al. 1999, Stone et al. 1999, 2003; Lambert et al. 2007, Walker et al. 2008, Albert et al. 2009). For example, in the state of New York between 1971 and 1997 there were at least 51 confirmed cases of death by hemorrhage with detection of rodenticides in tissues of wildlife (Stone et al. 1999). A surveillance program (1998 to 2001) reported that nearly half of the 265 raptors examined had detectable quantities of anticoagulant rodenticides in liver tissue, and these compounds were considered the cause of death in about 15% of these cases (Stone et al. 2003). Many of the incidents involved birds of prey, particularly great horned owls (*Bubo virginianus*) and red-tailed hawks (*Buteo jamaicensis*), that consume exposed or poisoned rodents. The global magnitude of secondary poisoning by rodenticides in birds is unknown, as most events are probably unnoticed or not reported.

A risk assessment by the U.S. Environmental Protection Agency (US EPA) identified several rodenticides that pose a significant risk to birds and non-target mammals (Erickson and Urban 2004), and subsequently some restrictions were placed on the sale, distribution, and packaging of brodifacoum, difethialone, bromadiolone, and difenacoum (US EPA 2008). This action may be offset by expanded use of other anticoagulant rodent-

cides, including diphacinone. The hazard of diphacinone to non-target organisms is inadequately characterized. Accordingly, sublethal responses (blood clotting time) and lethality were determined in northern bobwhite (*Colinus virginianus*), a species traditionally used in wildlife pesticide risk assessments, and also in the American kestrel (*Falco sparverius*), a well-studied toxicological model for raptorial species (Bardo and Bird 2009). Rather than using *ad libitum* dietary exposure in which food consumption can be highly variable, a controlled oral dosing regimen was employed to more accurately estimate dose-related sublethal and lethal effect thresholds. These and other data will ultimately assist in the development of a pharmacodynamic model of diphacinone in birds, and also in selection of efficacious baiting strategies that may mitigate risk to non-target species.

METHODS

Animals

Adult northern bobwhite were obtained from R & R Game Birds (Lamar, CO), housed individually in indoor pens (61 cm × 46 cm × 33 cm high) at the USDA National Wildlife Research Center, Fort Collins, CO (NWRC), maintained in a 12 hr light:12 hr dark photoperiod at 18-21°C, and provided food (Purina Game Bird Maintenance Chow[®] Product 5440, 12.5% protein, Denver, CO) and water *ad libitum*. Body weight of quail ranged from 149-224 g. American kestrels were propagated in the captive colony of the USGS Patuxent Wildlife Research Center, Laurel, MD (PWRC) (Porter

and Wiemeyer 1970), where they were maintained in outdoor flight pens (6.1 m × 2.4 m × 2.1 m high), and provided daily rations of either Classic Bird of Prey diet (Nebraska Brand, North Platte, NE) supplemented with Vionate® (Gimborn US, Atlanta, GA), dead mice (*Mus musculus*), or dead hatchling chickens (*Gallus gallus*), and water. Body weight of kestrels ranged from 98-132 g.

Acute Toxicity of Diphacinone

An acute oral toxicity test was conducted in which quail were gavaged with technical grade diphacinone (99% active ingredient) (Hacco, Inc. Randolph, WI) suspended in vegetable oil (Crisco®, Orville, OH). Doses ranging from 917 to 3,666 mg/kg body weight ($n = 9-10$ quail/dose; about equal sex distribution/dose) were selected through a stepwise process. Due to the low solubility of diphacinone, birds in 4 of the treatment groups received multiple doses within a 24-hr period (total amount administered: 1,033 and 2,065 mg/kg – 2 doses/day; 2,868 mg/kg – 3 doses/day; and 3,666 mg/kg – 4 doses/day). The heaviest bird in a dosage group received 1 ml of the suspension, and the remaining quail in that group received a fraction of the volume determined by their weight. Vegetable oil (vehicle) was administered 1-3 times to 9 quail that served as controls. Birds were observed twice daily for signs of toxicity for 14 days.

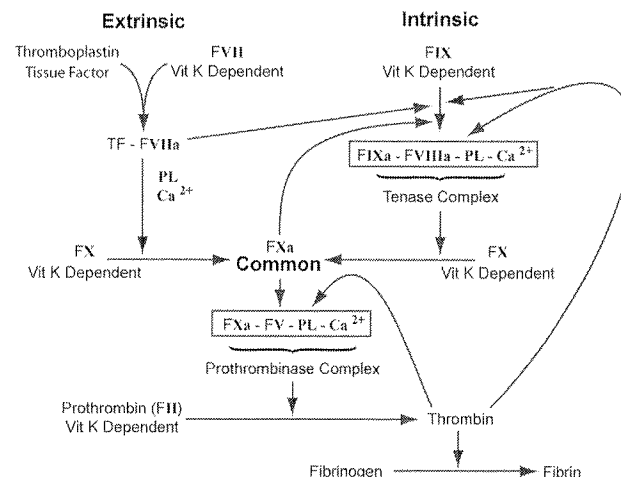
Using the results of the bobwhite acute toxicity test, range finding trials with kestrels were undertaken. Serious problems were encountered due to regurgitation of diphacinone. Through an iterative process, a dosing procedure was developed that minimized regurgitation. In September and October 2009, kestrels were moved from their flight pens to small outdoor cages (1.2 m × 0.8 m × 0.6 m high containing a rope perch, food tray, and water bowl) where they were housed individually and fed Classic Bird of Prey diet for at least a 10-day acclimation period. Following an overnight fast, small quantities of neat diphacinone (<200 mg/kg), freeze-dried bird of prey diet, and 5 µl FD&C Blue #1 food dye (to better detect regurgitation; McCormick & Co., Inc., Baltimore, MD) were loaded into a number 4 gelatin capsule (E. Lilly and Co., Indianapolis, IN) that was administered to the level of the proventriculus using a pilling device (modified pet piller, Jorjetsen Laboratories, Inc., Loveland, CO) and a plastic probe. The capsule was chased with 0.2 ml of distilled water by gavage, and the dosed kestrel was returned to its pen and immediately presented with a highly desired food item (i.e., dead chicken hatchling). By repeating this procedure 4 times within a 24-hr period, total daily dosages ranging from 35.1 to 675 mg/kg body weight were achieved ($n=19$ kestrels, with sexes divided near evenly among dosages, plus 4 controls that received capsules containing only the freeze-dried diet and blue dye). Pens were lined with kraft paper to monitor regurgitation, and kestrels were observed for overt signs of intoxication several times each day for a week.

Effects of Diphacinone on Clotting Time

Based upon the results of the acute toxicity test in quail, another study was conducted in which bobwhite

were gavaged with either vegetable oil (control, $n=6$), 434 mg diphacinone/kg body weight ($n=16$), or 783 mg diphacinone/kg ($n=16$). Quail were euthanized and immediately bled by cardiac puncture at 6, 12, 24, and 48 hrs post-dose, and controls were sacrificed and bled at 48 hrs post-dose. Blood samples (~0.5 ml) were collected into syringes containing 50 µl of 0.5 M EDTA, a suitable alternative to sodium citrate (Ceron et al. 2008). The samples were centrifuged, plasma was harvested and frozen at -80°C, and subsequently shipped to PWRC for analysis.

Using the aforementioned protocol from the kestrel acute toxicity trial, another kestrel diphacinone study was conducted in which a total of 50 mg/kg body weight was administered as divided doses over a 24-hr period. Controls were treated similarly except capsules did not contain diphacinone. At 48 hrs after administration of the final capsule, a 0.9-ml jugular venipuncture sample was drawn into a syringe containing 0.1 ml of 3.2% sodium citrate ($n=3$ diphacinone-treated and $n=3$ control kestrels), and blood samples were collected from the remaining birds ($n=3$ diphacinone-treated and $n=2$ controls) after 168 hrs. Blood samples were centrifuged and citrated plasma was frozen at -80°C for clotting time assays.



Adapted from Gentry 1993 and Thomson et al. 2002

Figure 1. Blood coagulation pathway in birds (adapted from Gentry 1993 and Thomson et al. 2002; F = Factor, PL = phospholipid).

One-Stage Prothrombin Time Assay

An excess of tissue factor and phospholipid (thromboplastin) interacts with plasma Factor VII to form an active complex, and through a cascade of reactions fibrinogen is eventually converted to fibrin which forms a clot (Figure 1). Crude chick hatchling thromboplastin (CHT) was prepared by the method of Quick as modified by Griminger et al. 1970 and Doerr et al. 1975. The CHT (50 mg) was suspended in 2,500 µl of 25 mM CaCl₂, and incubated at 42°C for 15 min with intermittent vortexing. Following centrifugation of the suspension (1,500 × g for 20 min), the supernatant was diluted (1:1) with 25 mM CaCl₂ (~220 µg protein/ml). Clotting time was determined using a BBL fibrometer (Becton Dickson & Co., Baltimore, MD). Plasma (100 µl) was incubated at 37°C

for 2 min, and the reaction was initiated by the addition of 200 μ l of diluted CHT. Intra-assay precision (mean coefficient of variation \pm standard deviation) for duplicate determinations of quail and kestrel plasma was $4.6 \pm 4.5\%$ ($n=30$) and $2.0 \pm 2.2\%$ ($n=11$), respectively. Inter-assay precision over the course of a year for human reference samples using Simplastin® (rabbit brain thromboplastin; Trinity Biotech, Berkeley Heights, NJ) was $2.9 \pm 2.2\%$ ($n=13$). When a quail or a kestrel plasma pool was diluted with 8.3 mM Na/K phosphate buffer (pH 7.2), clotting time was relatively stable at dilutions containing as little as 50% plasma, but increased at greater dilution.

Russell's Viper Venom Time (RVVT)

Russell's Viper venom (RVV) directly activates Factor X (but not Factor VII) in the common pathway of the clotting cascade (Figure 1). Reconstituted RVV Factor X activator (American Diagnostica, Stamford, CT) was diluted 1:10 with imidazole buffered saline (IBS; 0.0125 M imidazole 0.109 M NaCl, pH7.4) and maintained at room temperature. Plasma (100 μ l) was incubated at 37°C in a sample cup for 2 min, and 100 μ l of diluted RVV was added and incubated for 15 sec. The reaction was initiated with 100 μ l 25 mM CaCl_2 , and clotting time was determined (Triplett and Harms 1981a). Intra-assay precision for duplicate determinations of quail plasma was $6.5 \pm 13.5\%$ ($n=19$) and kestrel plasma was $3.4 \pm 4.1\%$ ($n=9$). Clotting time remained relatively stable when quail or kestrel plasma was diluted by as much as 60% with phosphate buffer, but RVVT increased dramatically at greater dilutions.

Thrombin Clotting Time (TCT)

This assay measures the time for conversion of fibrinogen to fibrin (Figure 1) using a standard thrombin solution (Triplett and Harms 1981b). The assay is an indicator of the amount of fibrinogen in the plasma sample, and insensitive to deficiency of vitamin K-dependent clotting factors. We used the AMAX Fibrinogen kit (Trinity Biotech) which includes bovine thrombin reagent and human fibrinogen reference material. A fibrinogen standard curve was prepared (65 to 520 mg/dL), and quail or kestrel plasma samples were diluted 1:10 with IBS. Diluted plasma (200 μ l) was incubated at 37°C in a sample cup for 2 min, and the reaction initiated by the addition of 100 μ l of thrombin reagent. Clotting time of the test sample was transformed to fibrinogen concentration (mg/dL) from the standard curve. Intra-assay precision for duplicate determinations of quail plasma was $5.7 \pm 6.8\%$ ($n=32$) and kestrel plasma was $1.8 \pm 2.2\%$ ($n=9$).

Statistical Methods

For the acute toxicity trial, the median lethal dose (LD_{50}) of diphacinone in bobwhite and kestrels was estimated using probit analysis (SAS Institute, Carey, NC). For sublethal dosing studies, prothrombin time, RVVT, and TCT were tested for homogeneity of variance (Fmax test) and normality (Shapiro-Wilk test, normal probability plot and descriptive statistics). The measurement endpoints were then compared by one-way analysis

of variance (ANOVA) in the quail study and by a 2×2 factorial ANOVA (dosage \times time) in the kestrel study. Tukey's HSD test was used as a mean separation procedure.

RESULTS

Acute Toxicity Studies

Survival of northern bobwhite was significantly related ($P < 0.0001$) to dose of diphacinone (survivors: 9 of 10 at 917 mg/kg; 8 of 9 at 965 mg/kg; 10 of 10 at 1,033 mg/kg; 7 of 10 at 2,065 mg/kg; 1 of 10 at 2,868 mg/kg; 0 of 10 at 3,666 mg/kg). Bobwhite receiving the greatest doses (2,868 and 3,666 mg/kg) succumbed within 1-3 days of exposure. Some dosed quail exhibited subcutaneous bruises on the breast and back regions which could reflect coagulopathy; however, there was no evidence of frank internal or external bleeding. All vehicle-dosed controls survived the 14-day trial. The LD_{50} estimate was 2,014 mg/kg (95% confidence interval 1,620-2,475 mg/kg), and the slope of the dose-response curve was steep (probit/ $\log_{10} \pm \text{SE} = 9.92 \pm 2.27$).

Survival of American kestrels was also significantly related ($P < 0.023$) to dose of diphacinone (survivors: 2 of 2 at both 35.1 and 52.7 mg/kg, 2 of 3 at 79.0 mg/kg, 0 of 3 at 118.6 mg/kg, 0 of 2 at 177.8 mg/kg, 0 of 1 at 200 mg/kg, 1 of 1 at 266.7 mg/kg, 0 of 2 at 300 mg/kg, 0 of 1 at both 450 and 675 mg/kg). Sample size for two intermediate doses was only 1, as a bird scheduled to receive 266.7 mg/kg died before the final capsule containing its divided dose could be administered. Birds scheduled to receive 600 and 900 mg/kg died before the final divided dose could be given, so the actual administered doses were 450 and 675 mg/kg. Kestrels that succumbed appeared to exhibit a progression of toxic signs (loss of balance on perch, standing on floor of pen rather than perch, non-reactive when approached, subdued behavior, appearance of tan urate deposits) and died between 10 to 48 hrs after receiving the initial capsule of the divided dose. Two kestrels (266.7 and 79.0 mg/kg doses) exhibited toxic signs, but appeared to recover by the third day of the trial. All controls survived the 7-day trial. The LD_{50} of diphacinone for kestrels was estimated to be 97 mg/kg (95% confidence interval 38-219 mg/kg), and the slope of the dose-response curve was 6.69 ± 2.94 probit/ \log_{10} .

Sublethal Exposure and Effects on Clotting Time

In the quail study, the 434 mg/kg dose was slightly greater than the lower 95% confidence limit of the LD_{01} , while the 783 mg/kg dose fell between the estimated LD_{01} and LD_{02} . Both of these doses prolonged clotting time when compared to controls, but the temporal response was highly variable (e.g., see scatter of prothrombin times in Figure 2). However, fibrinogen concentration in 5 samples as determined in the TCT assay was undetectable, and the volume of another sample was too small to determine fibrinogen concentration. The absence of fibrinogen in these samples suggest that they may have been collected improperly (viz., cardiac puncture of euthanized birds, partially clotted blood). Fibrinogen is generally in great excess, and its conversion to fibrin in the TCT assay is not influenced by vitamin K antagonists.

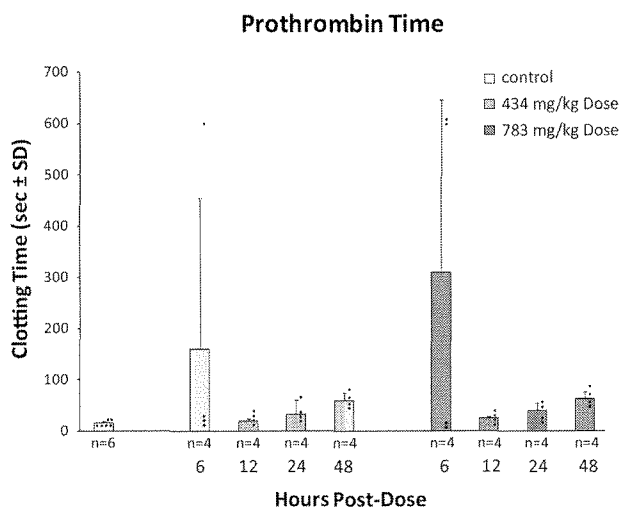


Figure 2. Prothrombin time (mean \pm standard deviation; \cdot = data point) of all quail gavaged with vehicle (control) or at 6, 12, 24, and 48 hrs following administration of diphacinone.

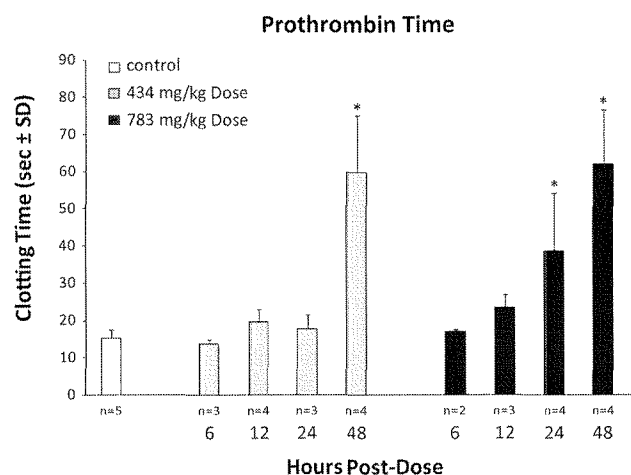


Figure 3. Prothrombin time of quail gavaged with vehicle (control) or diphacinone (6, 12, 24, and 48 hrs post-dose) with plasma fibrinogen concentration >60 mg/dL. * = significantly different ($P < 0.05$) than control.

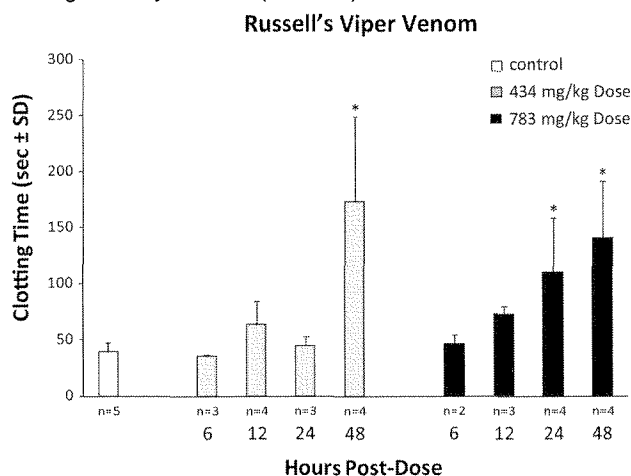


Figure 4. Russell's Viper venom time of quail gavaged with vehicle or diphacinone (6, 12, 24, and 48 hrs post-dose) with plasma fibrinogen concentration >60 mg/dL. * = significantly different ($P < 0.05$) than control.

When these 6 samples were excluded, the remaining samples contained more than 60 mg fibrinogen/dL (range: 63 to 254 mg/dL, $n=32$). For these remaining samples, prothrombin time and RVVT (Figures 3 and 4) were prolonged about three- to four-fold ($P < 0.05$) at 48 hrs after administration of 434 mg/kg, and similarly prolonged by 783 mg/kg at both 24 hrs and 48 hrs ($P < 0.05$), when compared to the control group.

In the kestrel study, the 50 mg/kg dose fell between the estimated LD_{10} and LD_{15} , and all birds survived the trial. However, one diphacinone-dosed kestrel was subdued on days 2 and 3, and before it was bled a subcutaneous hematoma was observed on its neck. A significant dose \times time interaction was detected by ANOVA for both prothrombin time ($P < 0.023$) and RVVT ($P < 0.027$). Diphacinone prolonged prothrombin time ($P < 0.036$) and RVVT ($P < 0.032$) in blood samples collected 48 hrs after administration of the final capsule of the divided dose, but after 7 days clotting times returned to control values (Figures 5 and 6). Fibrinogen concentration was detectable in all samples (range: 45 to 147 mg/dL) and did not differ ($P > 0.15$) among the 4 groups.

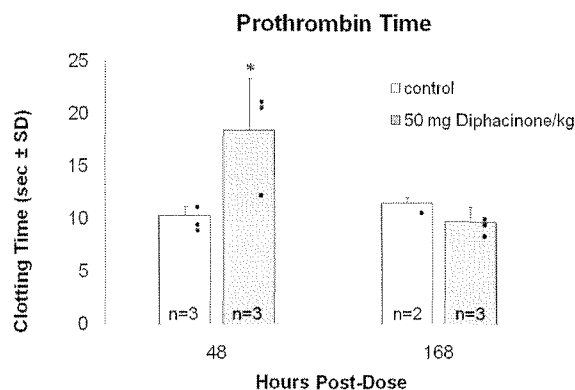


Figure 5. Prothrombin time of kestrels administered 4 capsules containing diphacinone (50 mg/kg over 24 hrs in divided doses) or capsules without rodenticide (control) at 48 and 168 hrs post-dose. * = significantly different ($P < 0.05$) than concurrent control.

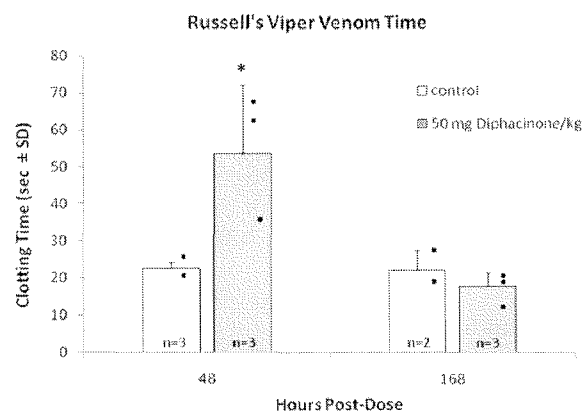


Figure 6. Russell's Viper venom time of kestrels administered 4 capsules containing diphacinone (50 mg/kg over 24 hrs in divided doses) or capsules without rodenticide (control) at 48 and 168 hrs post-dose. * = significantly different ($P < 0.05$) than concurrent control.

DISCUSSION

Diphacinone may be categorized as only slightly toxic (LD_{50} 2,014 mg/kg) to northern bobwhite using traditional categories that classify harm (Loomis 1978). A reliable diphacinone median lethal dose for bobwhite could not be estimated in a previous study (Campbell et al. 1991) as dosages were separated by a factor of 5, but inspection of the data suggest that the theoretical value fell between 400 and 2,000 mg/kg (US EPA 1998). Our estimated LD_{50} in quail is of the same order of magnitude as reported for mallards (*Anas platyrhynchos*; 3,158 mg/kg) (Erikson and Urban 2004). Based upon data from avian species commonly used in pesticide registration tests (viz., northern bobwhite, mallards), diphacinone appears to be less toxic to captive birds than to laboratory rats and domesticated mammals (range of estimated LD_{50} : 0.8 to 15 mg/kg), and to wild mammals (0.2 to 340 mg/kg), and its risk to wild birds would seemingly be minimal (reviewed in Erikson and Urban 2004, Eisemann and Swift 2006). However, results of the diphacinone acute toxicity test in American kestrels (LD_{50} 97 mg/kg) indicate that they are over 20 times more sensitive than bobwhite, and over 30 times more sensitive than mallards. Furthermore, a small dosing trial in which diphacinone-poisoned mice (*Peromyscus maniculatus*) were fed to great-horned owls (*Bubo virginianus*) and a saw-whet owl (*Aegolius acadicus*) also suggests that they are more sensitive than bobwhite (Mendenhall and Pank 1980). Notably, diphacinone has been linked to secondary poisoning in raptors (Stone et al. 1999, 2003), and in general, raptors are more sensitive to pesticides than other groups of birds (Wiemeyer and Sparling 1991, Vyas et al. 1998, Mineau et al. 1999). These findings indicate that extrapolation of diphacinone toxicity data from quail and mallards to other avian Orders (e.g., Falconiformes, Stringiformes) may be dubious, and protection of raptors may require substantial safety factors.

Some kestrels that survived dosing trials exhibited behavioral changes (e.g., 1 dosed at 266.7 mg/kg, and 1 of 3 dosed at 79.0 mg/kg) and prolonged clotting time (2 of 3 dosed at 50 mg/kg). Similarly, golden eagles (*Aquila chrysaetos*) fed muscle from sheep dosed with diphacinone (30 mg/kg) were weakened and debilitated and had prolonged prothrombin time (Savarie et al. 1979). In both of these studies animals recovered, but such behavioral and physiological deficits could affect survival of free-ranging birds.

Several coagulation assays were adapted that yielded short and precise clotting times with EDTA-treated plasma from quail and citrated plasma from kestrels. Using a thromboplastin extract from chick hatchlings, prothrombin time of untreated quail (mean \pm standard deviation: 15.2 ± 1.5 sec, Figure 3) and kestrels (10.6 ± 0.8 , Figure 5) was in the range of values reported for many species of domesticated and wild birds (Martin et al. 1994, reviewed in Powers 2000, Thomson et al. 2002, Morrissey et al. 2003, Rattner et al. 2009, Webster 2009). Using RVV that activates Factor X in the common pathway, clotting time of plasma from untreated quail (39.5 ± 8.9 sec, Figure 4) and kestrels (22.6 ± 2.8 , Figure 6) was slightly greater than reported in other avian species (~ 9 to 21 sec; Tahira et al. 1977, Timms 1977), although precision for

duplicate RVVT determinations and standard deviation of control samples with >45 mg fibrinogen/dL, seems acceptable.

Anticoagulant rodenticides inhibit vitamin K-dependent post-translational processing of clotting Factors II (prothrombin), VII, IX, and X (reviewed in Powers 2000), but do not affect the synthesis of fibrinogen. Fibrinogen deficiency resulting from improper sample collection and from pathophysiologic conditions (e.g., hepatic synthetic failure, disseminated intravascular coagulation) can prolong *in vitro* clotting time. Avian studies examining anticoagulant rodenticide toxicity have failed to determine if sample fibrinogen content supports *in vitro* clot formation. A concentration threshold that supports clotting has yet to be established for birds, and in the interim, we used 60 mg/dL in quail and 45 mg/dL in kestrels. A conservative diagnostic approach for anticoagulant rodenticide studies that evaluate vitamin K-dependent coagulopathies would entail a combination of assays, namely prothrombin time and/or RVVT, plus determination of fibrinogen (TCT) to rule out any nonspecific influence on clotting time.

In the present study, several quail plasma samples did not contain detectable quantities of fibrinogen, failed to clot in both the *in vitro* one-stage prothrombin time and RVVT assays, and thus were excluded from our evaluation of diphacinone on hemostasis in quail. Clotting time of samples with detectable fibrinogen was not affected at 6 and 12 hrs after administration of diphacinone, but was prolonged at 24 and 48 hrs post-dose. In kestrels, effects on clotting time were apparent at 48 hrs after administration of the final divided dose of diphacinone. This time course roughly corresponds with, and may precede, the onset of overt toxicity and lethality at greater dosage levels used in the acute toxicity studies. The lag time between dosing and development of coagulopathy reflects the half-life clearance of functional coagulation factors and the increasing circulation of des-carboxy, dysfunctional factors. Prothrombin time is used as an early indicator of anticoagulant rodenticide ingestion in domestic mammals (Mount and Feldman 1983), and is routinely measured to monitor coumadin anticoagulant therapy in humans (Spinler et al. 2005). Prolonged prothrombin times have been reported within days of 1) dietary exposure to the anticoagulant rodenticides diphacinone (golden eagles, Savarie et al. 1979; American crow, *Corvus brachyrhynchos*, Massey et al. 1997), warfarin (chickens, Veltmann et al. 1981) and brodifacoum (Japanese quail, *Coturnix coturnix*, Webster 2009), and 2) repeated gavage with pindone (wedge-tailed eagles, *Aquila audax*, bronzewing pigeons, *Phaps chalcoptera*, Port Lincoln parrots, *Barnardius zonarius*, black ducks, *Anas superciliosa*, Australian magpies, *Gymnorhina tibicen*, Martin et al. 1994).

In conclusion, diphacinone was found to be considerably more toxic to American kestrels than to northern bobwhite. A group of clotting assays were developed and applied for use in quail and kestrels that are sensitive, precise, linked to the pathogenesis of toxicity (and ultimately mortality), and together have applicability as biomarkers in both laboratory studies and field monitoring. These findings and assay methods will assist

in rodenticide hazard and risk assessments of secondary poisoning in non-target avian species.

ACKNOWLEDGEMENTS

The authors wish to thank Wayne C. Bauer and Mary E. Maxey for care of kestrels, Dr. Joann Beaver of Wildlife International, Ltd. and Drs. Nimish B. Vyas and Gary H. Heinz for suggestions related to dosing of kestrels, and Drs. Thomas M. Primus and Chrisi Yoder for assistance with the quail study. Guidance and suggestions on the development of the clotting time assays was graciously provided by Dr. Marjory B. Brooks of the Comparative Coagulation Section, Cornell University, and Dr. John A. Doerr of the University of Maryland. Drs. Charles Eason of Lincoln University, Nimish B. Vyas, and David J. Hoffman reviewed a draft of this manuscript. All animal procedures were approved by the Institutional Animal Care and Use Committees of the National Wildlife Research Center (NWRC), USDA and the Patuxent Wildlife Research Center (PWRC), USGS. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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TO: Transportation Water and Infrastructure Committee
 (Supervisor Candace Andersen, Chair; Supervisor Mary Piepho)

FROM: Patrick Roche, Principal Planner, Advance Planning Section *P. Roche*

DATE: May 2, 2013

SUBJECT: Request to Accept New Material under the 24 Hour Exception Provision of the Better Government Ordinance, Agenda Item #9, Draft Comment Letter on *Draft Plan Bay Area*

County Code Section 25-2.206 (Better Government Ordinance) requires that *"all such staff material must be distributed to the policy body and be made available to the public 96 hours before the scheduled meeting."* The Code allows the policy body, by a three-fourths vote, to waive these limits *"when, in its judgment, it is essential to do so, providing that the County Administrator, appropriate Department Head, or staff member furnishes to the Board of Supervisors or other policy body a written explanation as to why the material could not be provided to the Board or other policy body and the general public within the above time limits."*

The attached draft comment letter on *Draft Plan Bay Area* was completed after distribution of the May 2, 2013 Transportation Water and Infrastructure Committee agenda packet. Staff respectfully requests that you accept this draft comment letter for your consideration.

Attachment (1 item)

Draft Comment Letter on Draft Plan Bay Area

May 14, 2013

The Honorable Amy Rein Worth, Chair
Metropolitan Transportation Commission
101 Eighth Street
Oakland, CA 94607

Subject: Comment on *Draft* Plan Bay Area

Dear Commissioner Worth:

Thank you for the opportunity to comment on *Draft* Plan Bay Area. We appreciate ABAG's and MTC's continued efforts in developing the Sustainable Communities Strategy for the Regional Transportation Plan pursuant to Senate Bill (SB) 375.

As we understand it, the preferred scenario proposed under *Draft* Plan Bay Area can be simply summarized by its two main strategies:

- Jobs-Housing Connection Strategy - 80 percent of residential growth and 66 percent of job growth for the region through the year 2040 are to be focused in Priority Development Areas, which were adopted by cities and counties throughout the nine-county region; and,
- Preferred Transportation Investment Strategy - 86 percent of the region's transportation funding through the year 2040 will be devoted to maintaining the region's existing transportation system, and the balance of region's transportation funding will be directed to next-generation transit projects and other high performing

project, to programs aimed at supporting focused growth and reducing GHG emissions, and local road maintenance programs.

The Bay Area unquestionably faces significant challenges in reaching the targeted reductions of greenhouse gas (GHG) emissions of 7% from 1990 levels by 2020 and 15% by 2035 set for the region by the California Air Resources Board. We believe the *Draft Plan Bay Area* is a good first step in setting a regional course to reduce GHG emissions as mandated under SB 375. Overall, we agree that *Draft Plan Bay Area* places the proper emphasis on the following to achieve the necessary GHG emission reductions for the region:

1. Focusing growth in the areas of the region best suited to handle it, so that housing and jobs are closer to one another and more of the Bay Area's agricultural and open space lands remain intact.
2. Maintaining the region's existing transportation system and targeting expansion of a balanced transportation system in support of regional and local growth objectives.
3. Placing more emphasis in transportation performance in funding future transportation projects.
4. Making modest investments in innovative transportation programs to reduce GHG emissions.

While we believe that *Draft Plan Bay Area* establishes a good initial framework to reduce the region's GHG emissions as mandated under SB 375, we are concerned that unless actions are taken at the state and federal levels the implementation of Plan Bay Area's jobs-housing connection strategy and transportation investment strategy will be greatly hampered. We appreciate that *Draft Plan Bay Area* acknowledges this problem by identifying four legislative advocacy objectives that seek changes in state and federal law to support both the jobs-housing connection and transportation strategies.

To support the jobs-housing connection strategy under *Draft Plan Bay Area*, we agree that the State Legislature must take immediate steps to replace the \$1 billion per year funding stream in tax-increment financing that was available to cities and counties under redevelopment, which

was used to finance affordable housing projects, critical infrastructure improvements, and economic development projects. Development in Contra Costa County's four Priority Development Areas (PDAs) Area will not materialize at the levels projected under *Draft Plan Bay Area* unless there is a new, locally controlled funding tool, which replaces redevelopment, to finance affordable housing projects and infrastructure improvements within the PDAs.

We also agree that after four decades the California Environmental Quality Act (CEQA) the State Legislature needs to update CEQA to provide more streamline environmental review in order to promote the infill development opportunities within the PDAs as envisioned under jobs-housing connection strategy in *Draft Plan Bay Area*.

At the federal level, we agree that federal funding reductions under the U.S. Department of Housing and Urban Development's HOME Investment Program and the Community Development Block Grant (CDBG) Program have negatively impacted the ability of cities and counties to finance affordable housing projects. To support and increase the supply of workforce housing development, as called for in the jobs-housing connection strategy, the funding levels for these key federal programs , which have been relied upon to develop and finance affordable housing projects, need to be restored to their historic levels. Contra Costa County's ability to assist in financing the development of workforce housing projects, particularly in the County's four PDAs, is not sustainable without higher levels of support from the HOME Investment Program or the CDBG Program.

To support *Draft Plan Bay Area's* transportation investment strategy, we also agree that state legislative actions are needed. Since local taxes now generate about two-thirds of the state's transportation funding, actions are needed at the state level to preserve and support the "self-help" countywide transportation funding measures, such as Contra Costa County's Measure J. *Draft Plan Bay Area's* transportation investment strategy is financially sustainable in the near-term because voters in eight of the nine counties have approved countywide transportation sales tax funding measures; however, many these "self-help" funding measures will term out within the 30-year horizon of *Draft Plan Bay* raising concerns about the long-term financial viability of the Preferred Transportation Investment Strategy if they are not extended. We,

therefore, agree that that lowering the voter approval threshold for such “self-help” funding measures from the two-thirds supermajority to 55 percent would increase the likelihood of extending such funding measures that the region and state now rely upon to fund transportation improvements. Importantly, we also concur with *Draft Plan Bay Area* that should any new statewide transportation fund source be established by the legislature, or through voter approval, it must be constitutionally dedicated to transportation so as to protect the funds from being diverted for purposes other than transportation.

As a final note, we do have more specific comments regarding *Draft Plan Bay Area* which are listed below.

Specific Comments

1. Page 14, subheading entitled, “**Embodying local visions**” – It our observation that during many of the public meetings concerning Plan Bay Area there has been a fundamental misunderstanding among some members of the public regarding the plan’s relationship to the land use authority of cities and counties. With this in mind the language here, and elsewhere in the document, should be clear and emphatic that Plan Bay Area will in no way usurp or interfere with the exercise of local land use authority by cities and counties. This additional clarifying language should include citation to the plain language of SB 375, and the state legislature intent about the retention of local land use authority.
2. Pages 110-111, “**Communities of Concern**”, it is encouraging to note that communities of concern, which are those neighborhoods that could be considered disadvantaged or vulnerable in terms of both current conditions and potential impact of future growth have been identified in Equity Analysis for the *Draft Plan Bay Area*. However, in reviewing the “Communities of Concern” map at page 110, we note that within Contra Costa County there are several unincorporated communities that are not shown on the map, most notably the Town of Rodeo (which earlier on had been identified under the Communities of Concern map posted to MTC website). This may be due to the fact that

there are unincorporated pockets, which might qualify as a disadvantaged neighborhood, but are not identified as such because they sit within a large census tract that include other neighborhoods with higher household incomes skewing the overall household income data for the census tract (this also applies to rural, unincorporated communities that sit within very large census tracts). The section on Communities of Concern should acknowledge unincorporated communities which due to their size or location within a census tract might be missed from the Equity Analysis, and some attempt should be made to identify unincorporated communities potentially missed from the Equity Analysis.

Thank you in advance for considering these comments on *Draft Plan Bay Area*.

Sincerely,

FEDERAL D. GLOVER

Chair, Contra Costa County Board of Supervisors

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